Induction Of Tolerance

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Adaptive Immune System and Self Tolerance

- One of the hallmarks of the adaptive immune system is its ability to recognize a vast number of different antigens.
- This ability is a consequence of the large lymphocyte repertoire, in which each cell has a different antigen receptor generated by the process of somatic recombination.
- This process is able to produce an estimate of 10^{15} different lymphocyte clones, each with a different antigen receptor that can hypothetically recognize any naturally occurring structure.
- Since somatic recombination is a random process, it generates T cell clones that can recognize self-structures or self-peptides (auto-antigens).

- The mechanism used by the immune system in order to avoid a possible harmful immune response against an individual's own cells and tissues is known as immune tolerance which can be classified into central and peripheral tolerance.

Central Tolerance. Central tolerance occurs in the thymus and allows the deletion of a major percentage of autoreactive T cells.

- *A-Positive Selection.* After originating in the bone marrow, the early precursors of T cells enter the thymus and migrate into the cortex where most of the subsequent maturation events take place.
- *B-Negative Selection.* Double positive cells are programmed to undergo apoptosis by default unless they receive a “rescue signal” which is provided by cortical thymic epithelial cells (cTEC) that express self-peptide/major histocompatibility complex (MHC). Only thymocytes recognizing self-peptide/MHC complex with low avidity will receive the rescue signals and will continue with the maturation process.
- As a consequence of positive and negative selection, T cells that leave the thymus and populate peripheral lymphoid tissues are self-MHC restricted and tolerant to many autoantigens

- *Peripheral Tolerance.* some autoreactive T cells are able to bypass this control and exit the thymus . In the periphery, these autoreactive clones are able to induce autoimmune responses, generally in response to an inflammatory environment such as one triggered during infection, Peripheral tolerance mechanisms involve the *deletion* of activated effector T cells, *anergy* induction, *clonal exhaustion,* and active *regulation* of effectors T cells.
- Regulatory T cells (Tregs) mediate active regulation of the immune response preventing autoimmune and inflammatory diseases and restraining responses to infections of viral, bacterial, or parasitic origin.

Two different types of Tregs have been described:

- Natural CD4+CD25+Foxp3+ regulatory T cells (nTregs), which are generated in the thymus and regulate immune responses in the periphery, and
- inducible CD4+CD25+Foxp3+ regulatory T cells (iTregs) which develop in the periphery from naïve CD4+ T cells after exposure to antigens in a specific cytokine microenvironment, tolerogenic APCs, or immunosuppressive drugs

Dendritic cells

- Dendritic cells play an important role in establishing peripheral tolerance.
- These cells are found in mucosal and parenchymal tissues where they function as sentinels in search for pathogens and tissue injury.
- During infection and tissue damage, immature DCs (iDCs) are activated through different pathogen-associated molecular pattern (PAMP) receptors, which trigger the maturation of DCs.
- These DCs migrate to the draining lymph nodes where they acquire the capacity to activate naïve T cells.
- Internalization of self-antigens present in apoptotic cells by peripheral iDCs induces tolerance mechanisms such as the expansion of iTregs that control effector responses and protect cells and tissues from damage during pathogenic autoimmunity

Definition of “Tolerance”

- persistent survival of a transplanted allograft in the absence of continuing immunosuppressive therapy and an ongoing destructive immune response.
- Achieving functional tolerance in transplant recipients will mandate that specific allograft-destructive responses are “switched off” while the global immune response to pathogens and carcinogens remains intact.

Need for Tolerance in Clinical Transplantation

TABLE 23-1 **Immunosuppressive Agents Used in Solid-Organ Transplantation**

Class of Agent	Agent
Corticosteroid	Prednisone Methyl prednisolone
Antiproliferative	Azathioprine Mycophenolate mofetil Mycophenolate sodium
Calcineurin inhibitor	Cyclosporine Tacrolimus
mTOR inhibitor	Sirolimus Everolimus
Polyclonal antilymphocyte antibodies	ALG ATG
Monoclonal antibodies (with target)	Muromonab (CD3) Basiliximab (IL-2α receptor CD25) Alemtuzumab (CD52) Rituximab (CD20)
Costimulation blockade	Belatacept (LEA29Y – CTLA-4-Ig)

ALG, antilymphocyte globulin; ATG, antithymocyte globulin; CTLA-4, cytotoxic T lymphocyte antigen-4; IL, interleukin; mTOR, mammalian target of rapamycin.

Mechanisms of Tolerance to Donor Antigens

The mechanisms identified as responsible for inducing or maintaining tolerance to donor antigens include the following:

- • Deletion of donor-reactive cells centrally in the thymus as well as in the periphery
- • T-cell ignorance, or a state of T-cell unresponsiveness that is relevant to grafts placed at “immunologically privileged” sites such as the cornea or brain
- • Exhaustion, in which the ability of donor-reactive cells is eliminated as a result of overstimulation and cell death
- • Anergy, defined as a state of unresponsiveness that is refractory to further stimulation
- • Immunoregulation – an active process whereby the immune response to an allograft is controlled by populations of regulatory immune cells.

Regulation of Immune Responses

- Different populations of immune regulatory cells can play a role in controlling the immune response after transplantation, including Treg, regulatory B cells (Breg), myeloid-derived suppressor cells (MDSCs), DCs, and regulatory macrophages.

Mesenchymal Stromal Cells

- Mesenchymal stromal cells (MSCs) are a subpopulation of multipotent cells within the bone marrow that support hematopoiesis and possess immunomodulatory and reparative properties.
- Bone marrow-derived MSCs have the ability to migrate to sites of inflammation, including to an allograft.
- Once activated, MSCs can control the activity of T cells,B cells,DCs,NKs,and macrophages,either through direct cell– cell contact or indirectly through the release of soluble factors into the local microenvironment.
- MSCs have been shown to promote the generation of Treg cells *in vitro* and *in vivo* through mechanisms involving prostaglandin E2, TGF-β, and cell–cell contact.

- In transplantation, retrieval and transplantation of the allograft inevitably result in ischemia and reperfusion injury creating an inflammatory microenvironment within the graft.
- The recruitment of MSCs to the graft in the early posttransplant period could potentially lead to the conversion of T cells, also recruited into the allograft, into Treg cells.
- The immunomodulatory properties of MSCs on B-cell function could also contribute to suppressing graft rejection by inhibiting alloantibody production.

Information from Analyzing Tolerant Recipients

- Operational tolerance remains a relatively rare event in the clinical setting.
- A small number of bone marrow transplant recipients who subsequently required a renal transplant were transplanted with a kidney from their bone marrow donor.
- In these cases, long-term immunosuppression was unnecessary because the recipient was already unresponsive to the donor alloantigens as a result of the allogeneic chimerism that developed after the successful bone marrow transplant.
- Clinical reports of patients exhibiting spontaneous tolerance to an allograft, after withdrawal of immunosuppression in the absence of a bone marrow transplant, are still infrequent but are increasing in number.
- By studying these patients in depth, it would be possible to define a molecular signature of tolerance in immunosuppression-free kidney and liver transplant recipients

- Discontinuation of immunosuppression by the clinical team as a consequence of the side effects of immunosuppression or non-adherence/ compliance or as a result of weaning of immunosuppression based on a clinical protocol (particularly in liver transplant recipients).
- liver “protects” other organs that are transplanted alongside it from the full force of the rejection response – the so-called liver effect.
- A number of mechanisms have been proposed to account for the liver effect, including; for example, the large antigen load delivered by the liver itself, the presence of a large number of passenger leukocytes that could result in the deletion of donor-reactive cells, and the establishment of long-lasting microchimerism in some recipients as well as the production of soluble MHC class I by the liver.

- Estimates vary, but in the order of 25–60% of liver transplant recipients appear to have the potential to be weaned from immunosuppression without the risk of rejection.
- Analysis of the peripheral blood from immunosuppression- free liver transplant recipients revealed an increase in CD25+CD4+ T cells with regulatory activity *in vitro* as well as a skewing of other T-cell subsets, notably γδ T cells, compared to the profiles found in age-matched controls.
- Interestingly, FOXP3+ cells were also found to be a prominent component of lymphocytes infiltrating the liver allografts.
- Further analysis using transcriptional profiling demonstrated that there was a molecular signature of tolerance associated with tolerance in immunosuppression-free liver transplant recipients.

- Two independent studies, one in Europe and the other in the United States, found that there was a signature of tolerance in immunosuppression-free kidney transplant recipients that could be cross-validated between the two cohorts of patients studied.
- Interestingly, the signature of tolerance in kidney transplant recipients was distinct from that identified in immunosuppression free liver transplant recipients.
- Gene expression analysis demonstrated that there was a pattern of gene expression associated with immunosuppression- free graft survival, nine of which were found to be most significant.

STRATEGIES WITH THE POTENTIAL TO INDUCE IMMUNOLOGIC TOLERANCE TO AN ALLOGRAFT

- Data emerging from the analysis of samples from patients enrolled in tolerance induction protocols suggest that no single mechanism can account for or dominate the tolerance induction or maintenance process in transplant recipients .

Mixed Chimerism

- The coexistence of stable mixtures of donor and recipient cells resulting in a state of allospecific tolerance is an idea that was initially restricted to the field of bone marrow and HSC transplantation.
- A small number of bone marrow transplant recipients who subsequently required a renal transplant, and were transplanted with a kidney from their bone marrow donor, exhibited tolerance to donor alloantigens.
- These cases provided a foundation for the development of non-myelo ablative conditioning protocols wherein donor bone marrow cells are introduced into recipients under conditions allowing for the development and maintenance of macrochimerism and long term allograft survival.

Mixed Chimerism as a Strategy to Induce Allograft Tolerance.

- To be considered mixed chimerism, donor cells in the blood must represent more than 1% of the total cells as measured by flow cytometry.
- To induce a state of mixed chimerism, it is necessary to perform a conditioning treatment in order to allow donor HSC bone marrow acceptance.
- The allogeneic BMT generates a new source of T cells and DCs that induces a relearning of the "new self " state, depleting the possible T cell clones that recognize both allo and autoantigens

Many different approaches have been used to achieve macrochimerism:

- Total lymphoid irradiation alone or in combination with bone marrow infusion has been shown to be effective at inducing tolerance in some recipients in rodents, primates, and human patients.
- Total lymphoid irradiation and antithymocyte globulin after transplantation resulted in the engraftment of donor HSC.

Tolerance Induction after Kidney Transplantation

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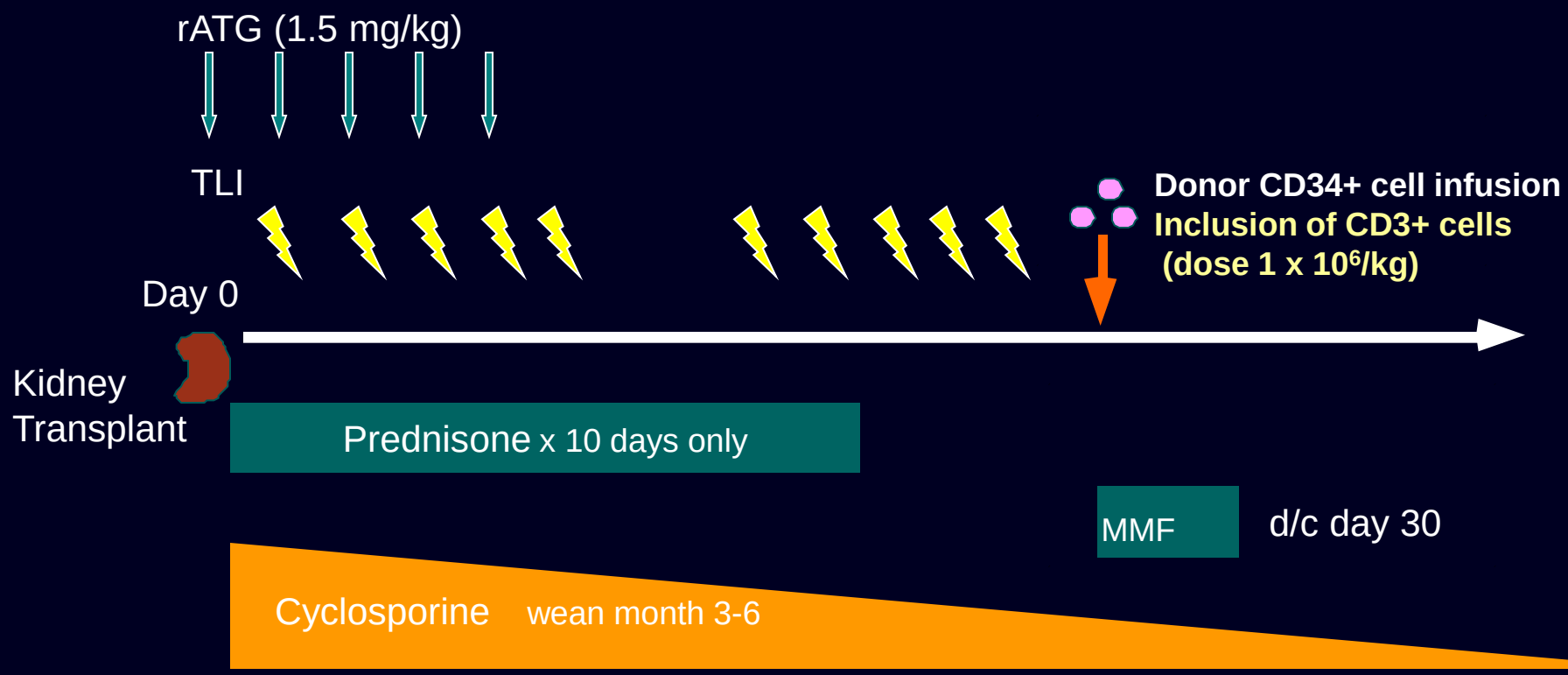
HLA-matched Combined Kidney/Hematopoietic Cell Transplantation Protocol

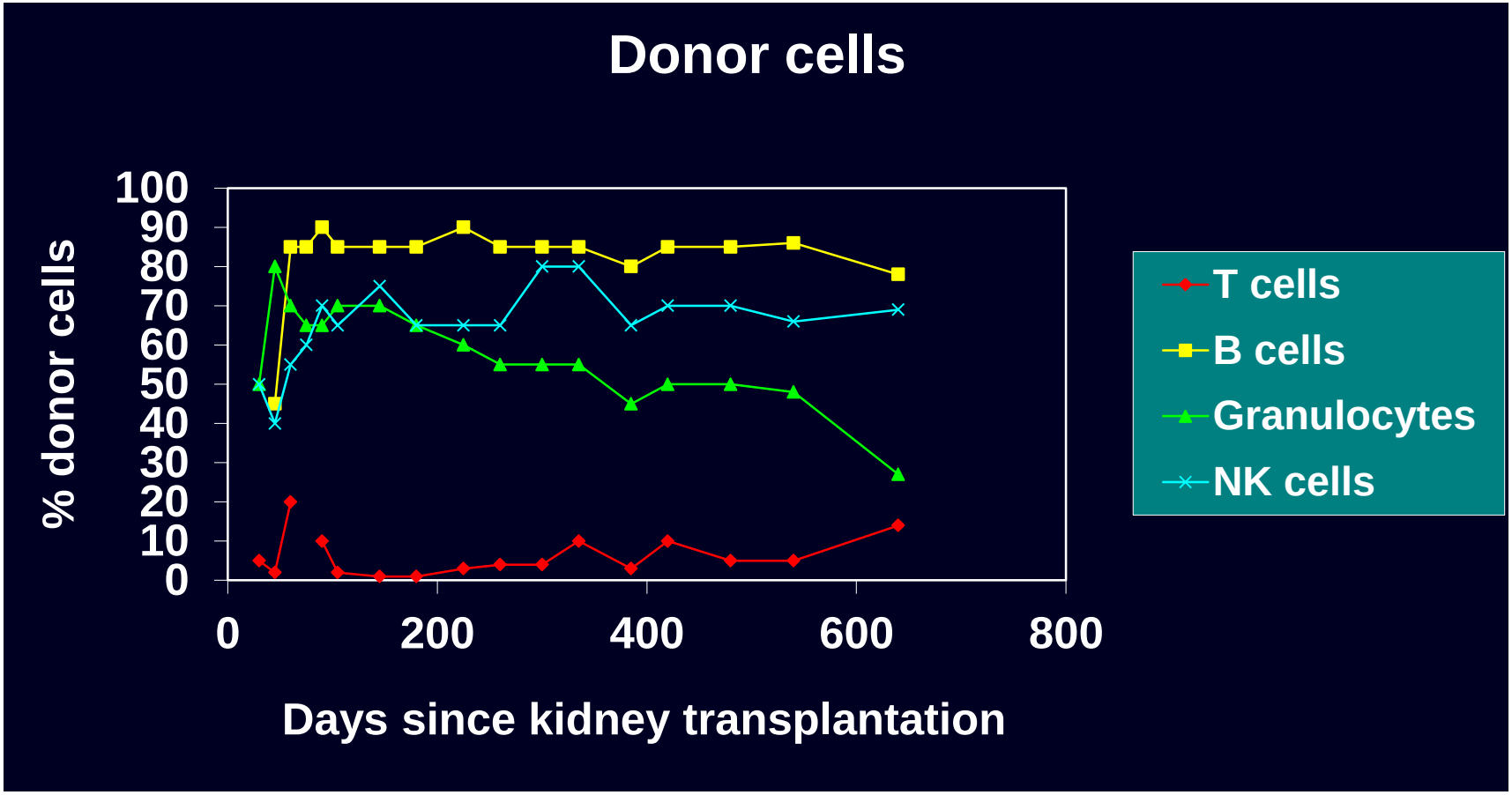


Withdraw immunosuppression if:
-stable macrochimerism
-no evidence of rejection
-no GVHD

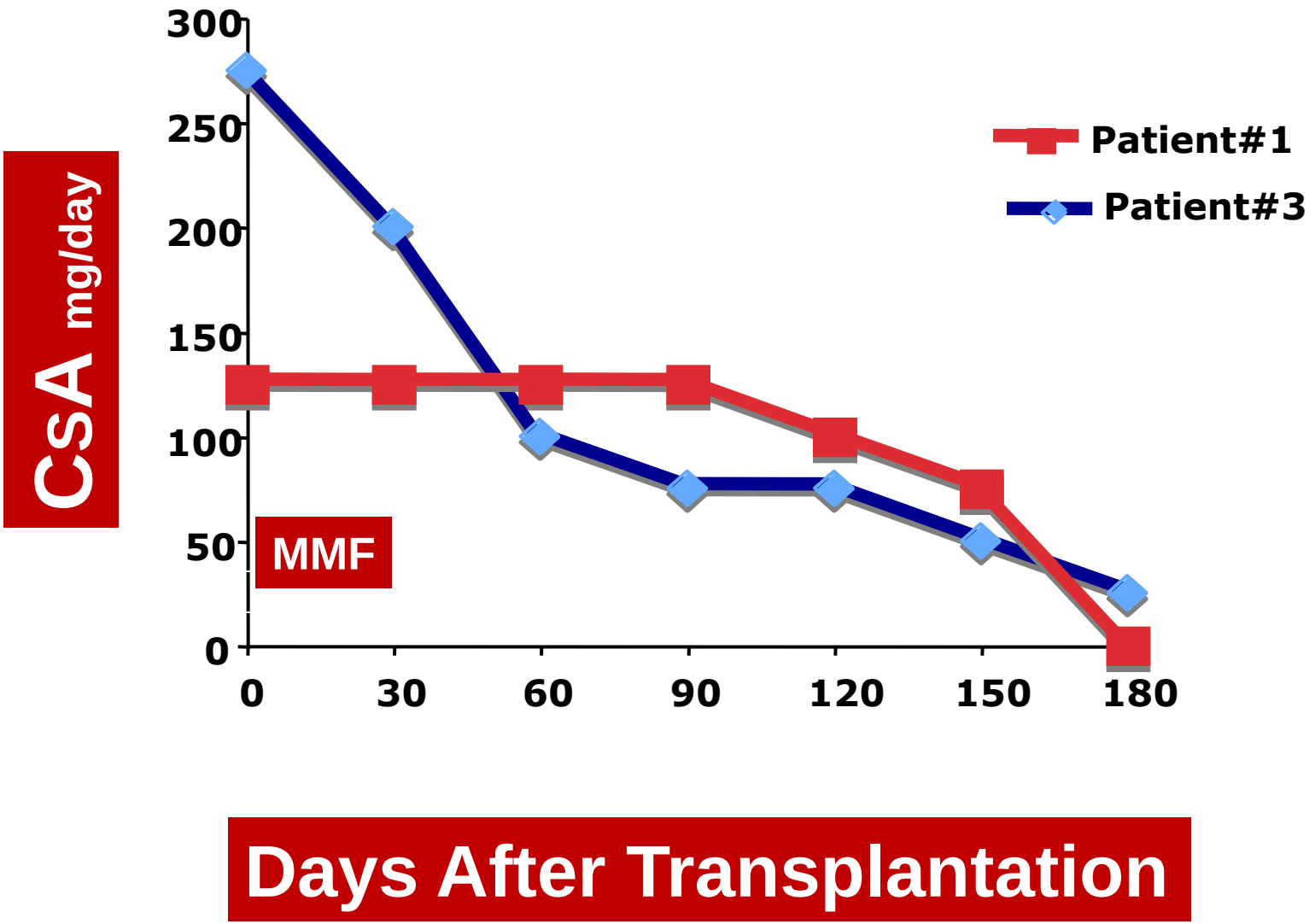
Subject Characteristics

Patient #	Age/ Gender	ESRD cause	HLA match	Relation to donor	$CD34^+$ dose ($x10^6$/kg)	$CD3^+$ dose ($x10^4$/kg)
1	48 M	?	6	brother	10	100
2	39 F	FSGS	6	sister	5	100
3	24 M	dysplasia	6	brother	5-10	100

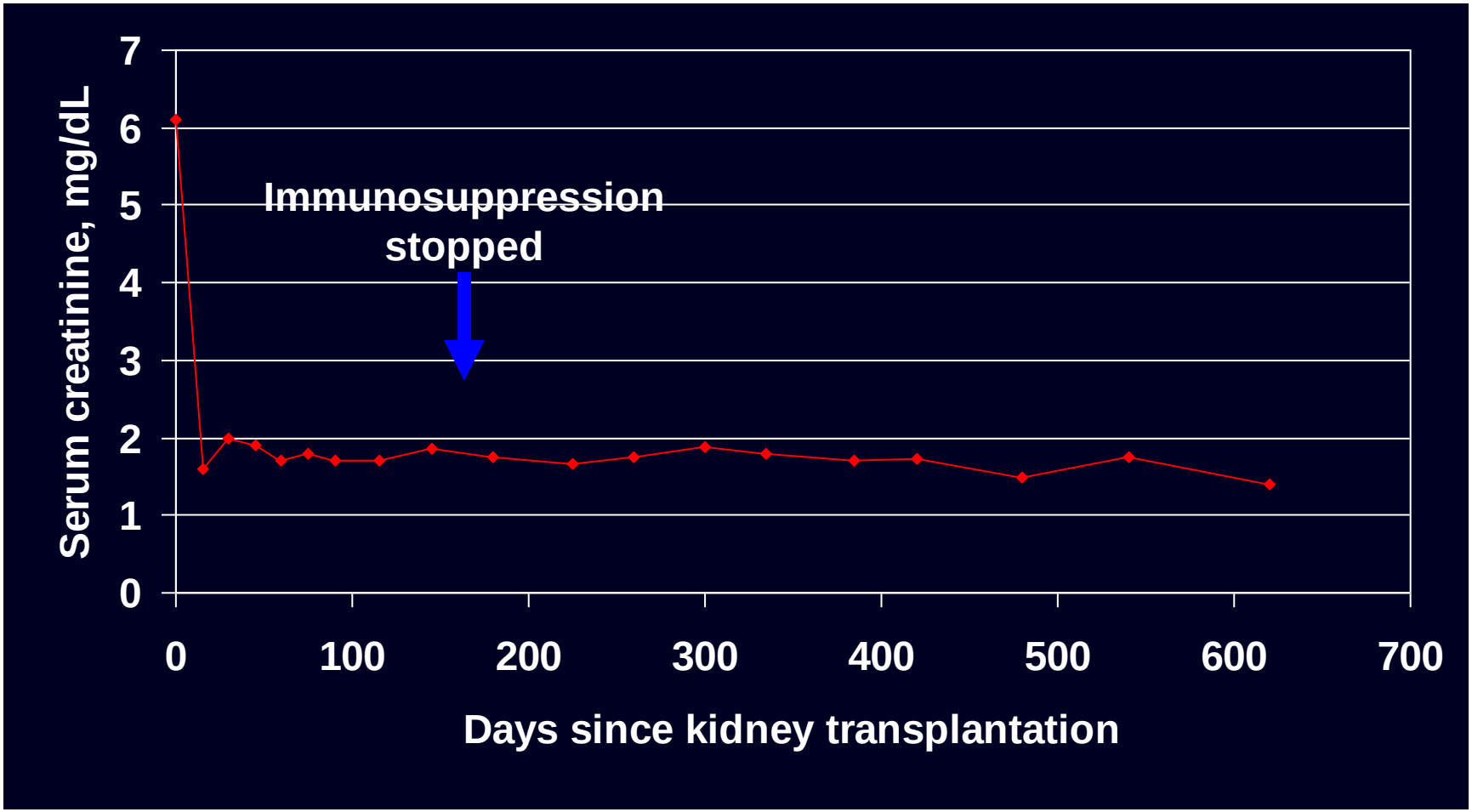
Chimerism
Subject # 1



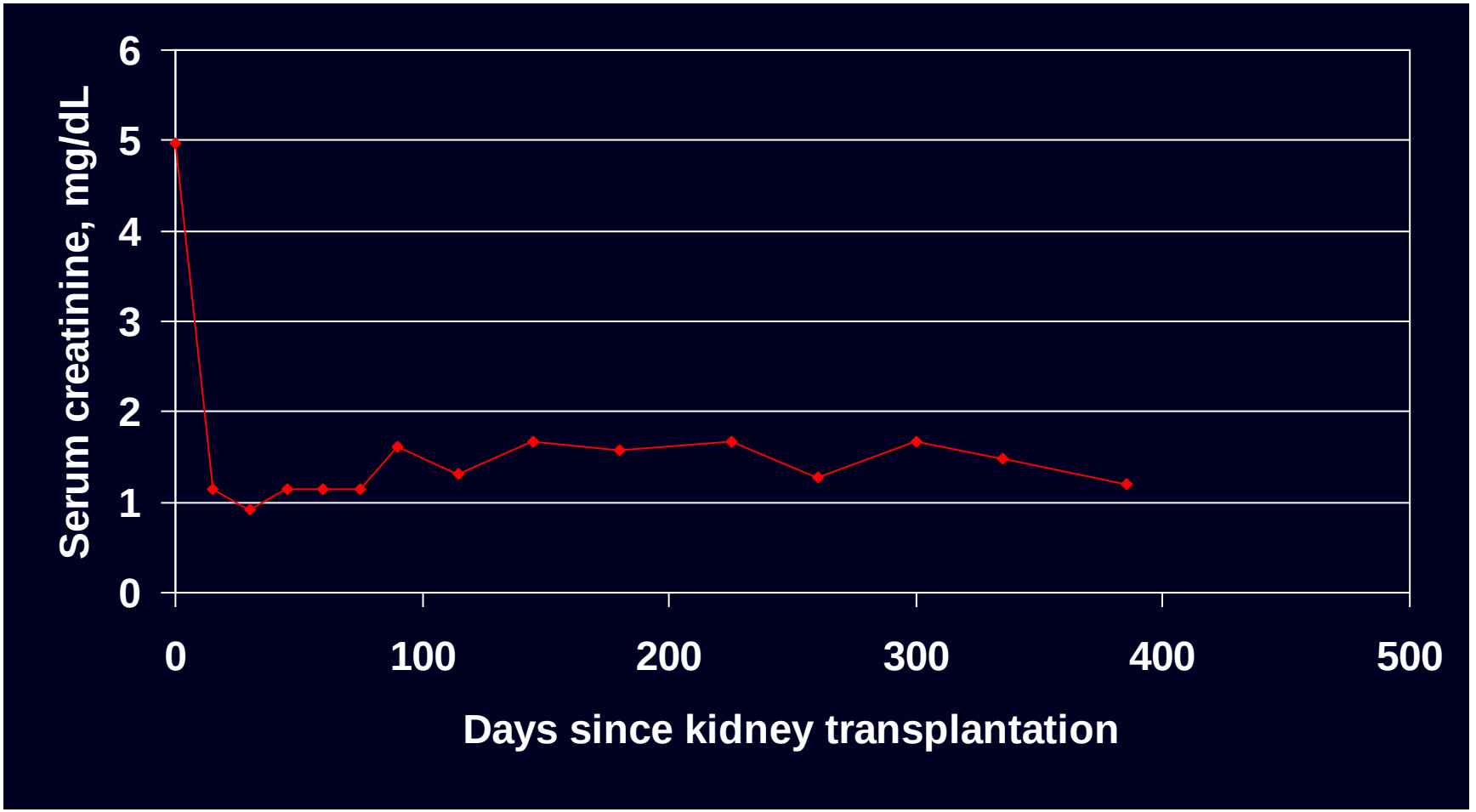
Drug Taper


CsA mg/day
300
250
200
150
100
50
0
MMF
0
30
60
90
120
150
180
Patient#1
Patient#3
Days After Transplantation

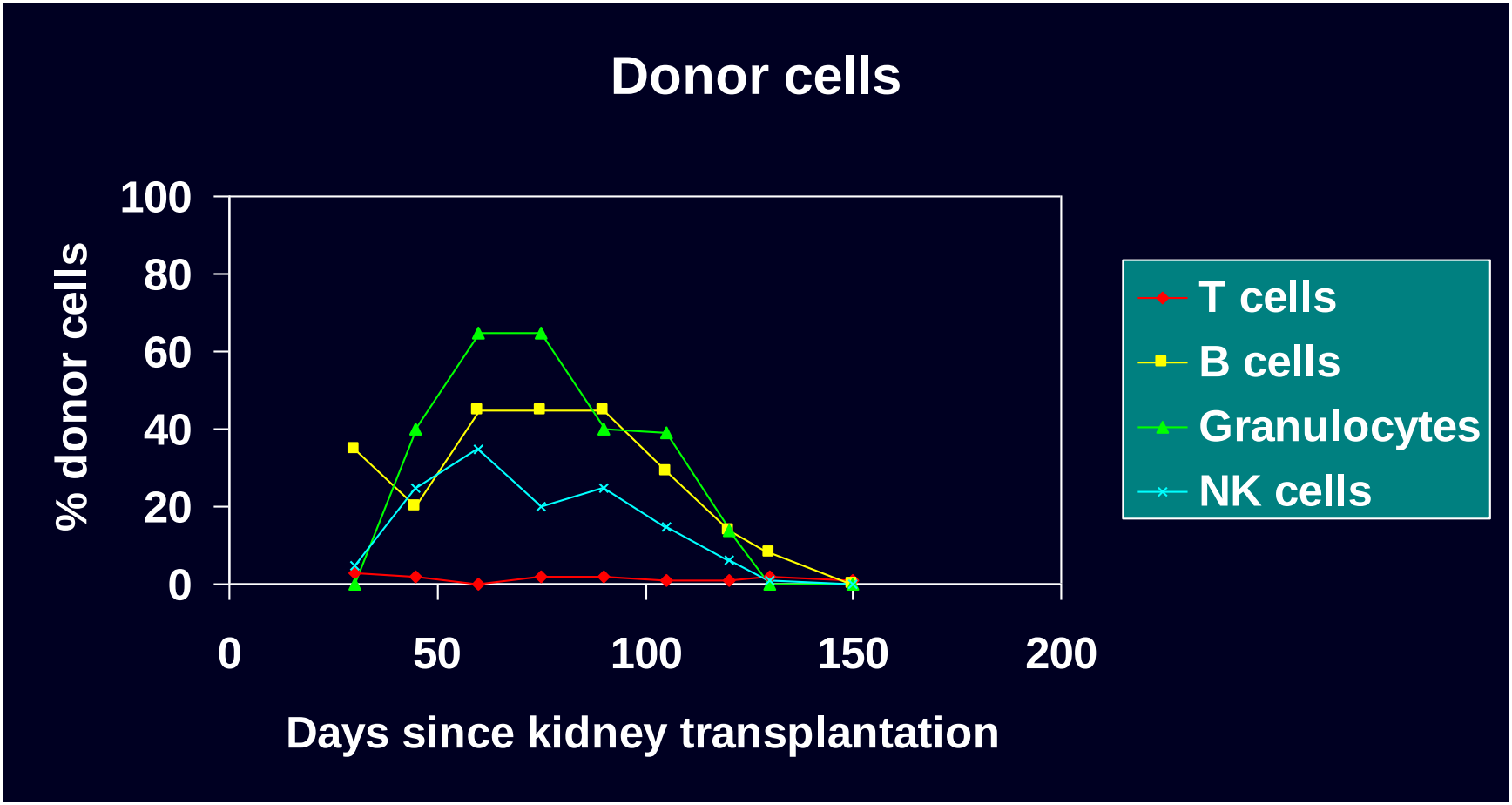
Allograft Function
Subject # 1



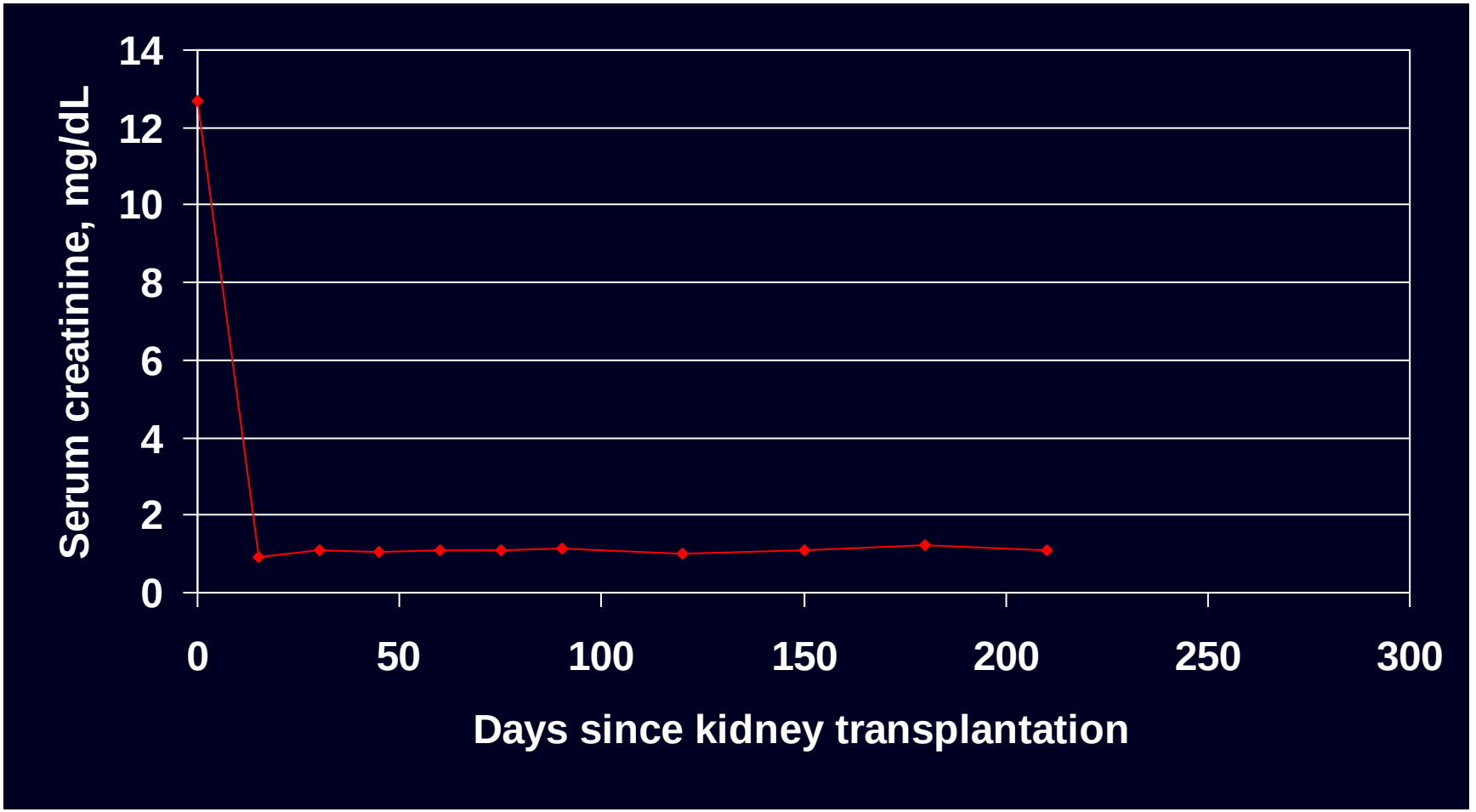
Allograft Function
Subject # 2



Chimerism
Subject # 3



Allograft Function
Subject # 3



Costimulation Blockade

- The activation of a T cell is dependent upon multiple signals.
- When antigen recognition occurs in the absence of costimulation, T cells become anergic or undergo apoptosis.
- Monoclonal antibodies and recombinant fusion proteins targeting costimulatory molecules are capable of inducing unresponsiveness to donor antigens *in vivo* by allowing antigen recognition to take place in the absence of costimulation.

The B7(1/2):CD28/CTLA-4 Pathway

- CD80 (B7-1) and CD86 (B7-2) are expressed as cell surface molecules by APCs and are responsible for delivering additional or second signals to T cells when they interact with their ligands CD28 and CD152 (CTLA-4).
- CD28 is expressed constitutively by T cells, while CD152 is only expressed later in the activation response.
- CD86 appears to interact preferentially with CD28 and may be the most important ligand for T-cell activation.
- CD80 may bind preferentially to CD152.
- In contrast to CD28, CD152 negatively regulates T-cell activation when it engages its ligand on the APC.
- Thus targeting CD28 will inhibit T-cell activation, whereas targeting CD152 will potentiate T-cell activation.

- Both approaches are of interest in different contexts, as demonstrated by the development of CTLA-4-Ig for preventing allograft rejection and the development of anti-CD152 as a cancer therapy.
- When CTLA-4-Ig, an immunoglobulin fusion protein of CTLA-4, was produced, it was shown to inhibit graft rejection in xenogeneic and allogeneic systems.
- In rodent models, CTLA-4-Ig therapy alone was found capable of inducing tolerance to the graft.
- However, this effect did not translate to primate models where CTLA-4-Ig monotherapy was not found to induce long-term graft survival.
- A mutated form of CTLA-4-Fc, LEA29Y or belatacept, was found to be a more potent inhibitor of allograft rejection than the native molecule, in primate renal transplant models

- Belatacept differs from CTLA-4-Ig by two amino acid sequences, which confers an approximately twofold greater binding capacity to CD80 and CD86.
- Belatacept has been approved for clinical use for the indication of prophylaxis of organ rejection in adult kidney transplant recipients (by the FDA in June, 2011 and EMA in April, 2011) when used in conjunction with basiliximab induction, mycophenolate mofetil, and corticosteroids as maintenance immunosuppression.

LEUKOCYTE DEPLETION AT THE TIME OF TRANSPLANTATION

- Many tolerance induction strategies that have been investigated in small- and large-animal studies result in the depletion of leukocytes (antithymocyte globulin, anti-CD52) or T cells (anti-CD3 with or without immunotoxin, CD2, CD4, and CD8) appears to be sufficient in some situations for tolerance to develop and be maintained in the long term.
- However, in humans, leukocyte depletion with the anti-CD52 monoclonal antibody alemtuzemab in combination with immunosuppressive drugs is not sufficient to induce operational tolerance, but can enable minimization of immunosuppression to control residual donor-reactive cells.

CELL THERAPY

- Cellular therapies using Treg, regulatory macrophages, and MSCs to suppress rejection or GVHD are being developed for use in clinical transplantation as a strategy to promote the development of specific unresponsiveness.

Treg Cell Therapy

- Tregs have been infused into bone marrow transplant recipients with the objective of limiting GVHD.
- Expanded Tregs were used to treat two patients who developed GVHD following bone marrow transplantation, with clinical improvement demonstrated in both patients.
- In organ transplantation, the ONE study, a multicenter phase I/II study funded by the European Union FP7 program, will investigate the safety of infusion of *ex vivo* expanded nTreg cells into kidney transplant recipients.

Regulatory T-Cell Therapy in the Induction of Transplant Tolerance: The Issue of Subpopulations

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Abstract. Clinical tolerance induction to permit minimization or cessation of immunosuppressive drugs is one of the key research goals in solid organ transplantation. The use of ex vivo expanded or manipulated immunologic cells, including $CD4^+CD25^{hi}FOXP3^+$ regulatory T cells (Tregs), to achieve this aim is already a reality, with several trials currently recruiting patients. Tregs are a highly suppressive, nonredundant, population of regulatory cells that prevent the development of autoimmune diseases in mammals. Data from transplanted humans and animal models support the notion that Tregs can mediate both induction and adoptive transfer of transplantation tolerance. However, human Tregs are highly heterogeneous and include subpopulations with the potential to produce the proinflammatory cytokine interleukin-17, which has been linked to transplant rejection. Tregs are also small in number in the peripheral circulation, thus they require ex vivo expansion before infusion into man. Selection of the most appropriate Treg population for cell therapy is, therefore, a critical step in ensuring successful clinical outcomes. In this review, we discuss Treg subpopulations, their subdivision based on nonmutually exclusive criteria of origin, expression of immunologic markers and function, availability in the peripheral blood of patients awaiting transplantation, and their suitability for programs of cell-based therapy.

Keywords: Regulatory T cells, FOXP3, Transplantation, Hemodialysis, Interleukin-17, Clinical trial, Subpopulation.

(*Transplantation* 2014;98: 370–379)

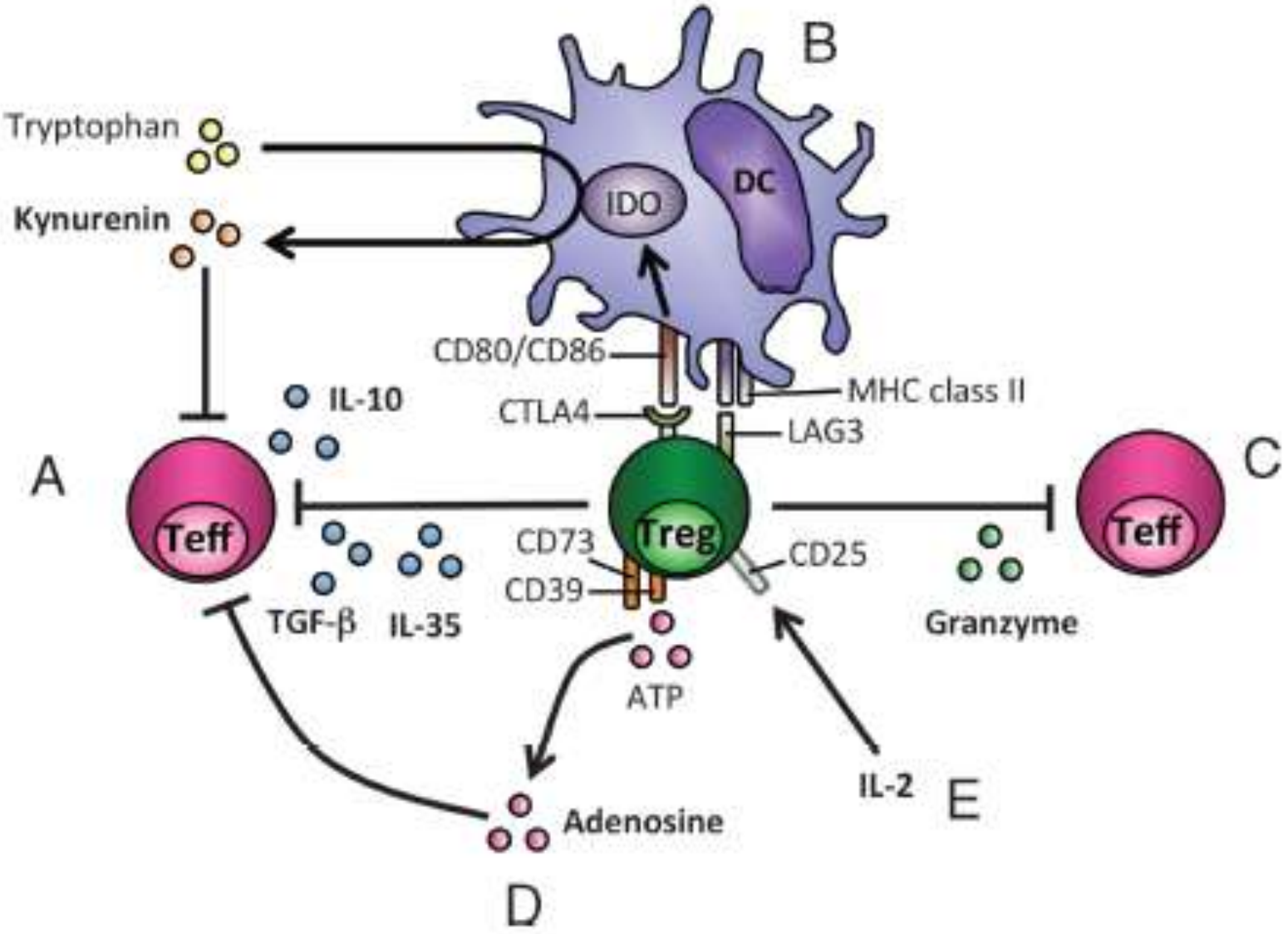
B
Tryptophan
Kynurenin
IDO
DC
CD80/CD86
MHC class II
CTLA4
LAG3
IL-10
A
Teff
Treg
C
Teff
CD73
CD39
CD25
TGF-β
IL-35
Granzyme
ATP
IL-2
E
Adenosine
D

TABLE 2. Clinical trials of Tregs published to date

Context	Trial protocol	Outcomes	Reference
GvHD after BMT	Two patients with GvHD, one acute, the other chronic, were administered ex vivo expanded donor Tregs. Prospective, uncontrolled study Single dose 10^5 cells/kg for patient with chronic GvHD Three doses of 3×10^6 cells/kg for patient with acute GvHD	The patient with chronic GvHD showed sustained clinical improvement. The patient with acute GvHD showed initial improvement, then disease progression, resulting in death. No adverse effects of Treg treatment were reported	(26)
UCB transplantation	23 patients receiving UCB transplantation also received ex vivo expanded third party UCB Tregs Prospective uncontrolled study $1–30\times10^5$ cells/kg after UCB	Patient receiving Tregs showed lower incidence of acute grade II–IV GvHD, with no toxicity associated with transfusion of Tregs nor increased infective episodes.	(27)
HLA-haploidentical HSCT	28 patients undergoing HSCT were given unexpanded donor Tregs before Teffs, without further immunosuppression Prospective uncontrolled study $2–4\times10^6$ Tregs/kg given 4 days before Teffs and $CD34^+$ cells	Tregs prevented development of GvHD in 26 patients; two developed acute GvHD; no patient development chronic GvHD. Tregs enhanced HSCT engraftment without impairing immunity or graft versus leukemia effect. There was a lower incidence of CMV reactivation than historical controls.	(28)
T1DM	10 children with T1DM within 2 months of diagnosis were given ex vivo expanded autologous Tregs; 10 controls were untreated with Tregs. Prospective controlled trial for four patients received 10×10^6 cells/kg; six patients had 20×10^6 cells/kg	Two patients were insulin-free 6 months later, eight patients still required insulin. Plasma C-peptide was increased in those receiving Tregs, indicating greater insulin production. There was no toxicity reported and no excess infective episodes.	(29)

GvHD, graft versus host disease; BMT, bone marrow transplantation; UCB, umbilical cord blood; HLA, human leukocyte antigen; HSCT, hematopoietic stem cell transplantation; T1DM, type 1 diabetes mellitus; CMV, cytomegalovirus.

TABLE 3. Ongoing and unpublished clinical trials using cell-based therapies to induce clinical tolerance

Clinical trial	Context
The "ONE" study	Cell-based therapy in the context of renal transplantation; multicenter, European Union funded. Participating centers and cell product administered are shown:
	King's College London, UK—Polyclonally expanded Tregs
	Oxford, UK—Polyclonally expanded Tregs
	University of California, San Francisco, CA—Alloantigen-specific Tregs
	Massachusetts General Hospital, Boston, MA—Anergic regulatory T cells
	CHU de Nantes Hotel-Dieu, France—Tolerogenic recipient dendritic cells
	Charite, Berlin—Polyclonally expanded Tregs
	Regensburg, Germany—Regulatory macrophages *and polyclonally expanded CD45RA$^+$ Tregs*
	Ospedale San Raffaele, Italy—Tr1 cells
The "ThRil" trial	*Treg-based cell therapy in the context of liver transplantation. Single-center trial, based at King's College London*
Edinger et al.	*Treg-based cell therapy to suppress GvHD after bone marrow transplantation. Single-center trial, based at Regensburg, Germany*
Blazar et al.	*Treg-based cell therapy to suppress GvHD after HSCT using HLA-matched sibling donors. Single-center trial, university of Minnesota*
Roncarolo et al.	Induced Tr1 cells used on patients with high risk malignancies receiving CD34$^+$ cells from a haplo-identical donor. Tr1 cells infused in the absence of immunosuppression after mononuclear cell engraftment as a donor lymphocyte infusion. Single-center trial, Milan, Italy
Bluestone et al.	*Treg-based therapy for adult patients with type 1 diabetes mellitus. Single-center trial, University of California, San Francisco, CA*
Salomon et al.	*Treg-based therapy for the treatment of uveitis. Single-center trial, Paris, France*
Yamashita et al.	*Treg-based therapy in the context of liver transplantation. Single-center trial, Sapporo Japan*

Treg-based protocols are italicized. GvHD, graft versus host disease; HSCT, hematopoietic stem cell transplant.

Regulatory Macrophage Cell Therapy

- Regulatory macrophages isolated from the organ donor have been administered intravenously to two living donor kidney transplant recipients with no deleterious impact on graft function, enabling tacrolimus immunosuppressive therapy to be reduced within the first 24 weeks after transplantation.
- A follow-up study using regulatory macrophages in kidney transplant recipients will be performed as part of the ONE study.

Mesenchymal Stromal Cell Therapy

- This form of cell therapy is still at an early stage of development.
- Not all clinical studies using MSCs to modulate immune reactivity have reported positive data. The activation status of the MSCs at the time of infusion may explain these inconsistencies.
- In kidney transplantation, infusion of autologous MSCs resulted in a lower incidence of acute rejection, a decreased risk of opportunistic infection, and better estimated renal function at 1 year posttransplantation.

Tolerance: One Transplant for Life

Tatsuo Kawai,[1] Joseph Leventhal,[2] Joren C. Madsen,[1,3] Samuel Strober,[4] Laurence A. Turka,[1,3,5,7] and Kathryn J. Wood[6]

Abstract. Recently, The Transplantation Society convened a workshop to address the question, "What do we need to have in place to make tolerance induction protocols a 'standard of care' for organ transplant recipients over the next decade?" In a productive 2-day meeting, there was wide-ranging discussion on a broad series of topics, resulting in five consensus recommendations as follows: (1) establish a registry of results for patients enrolled in tolerance trials; (2) establish standardized protocols for sample collection and storage; (3) establish standardized biomarkers and assays; (4) include children 12 years and older in protocols that have been validated in adults; and (5) establish a task force to engage third-party payers in discussions of how to fund tolerance trials. Future planned workshops will focus on progress in implementing these recommendations and identifying other steps that the community needs to take.

Keywords: Tolerance, Transplant, Life.

(*Transplantation* 2014;98: 117–121)


Thank You!